

SHELL PERMEABILITY AND DESICCATION PHYSIOLOGY OF THE FRESHWATER SNAIL *BULINUS (BULINUS) TROPICUS* (KRAUSS)

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ABSTRACT

The water permeability rate of the shell wall of *Bulinus tropicus* (Krauss) was determined with tritiated water to be 1.74 ± 1.06 μl water per 24 h. The osmotic pressure of the haemolymph increased three-fold to 300 ± 16 mOsm l^{-1} after 60 days at 85% r.h. of the air. The [Na] of the snail tissues increased from 38 ± 9.0 mMol l^{-1} to 68 ± 11.0 mMol l^{-1} after 60 days at 85% r.h., but at 96% r.h. the increase changed from 40 ± 7.0 mMol l^{-1} to 55 ± 6.5 mMol l^{-1} . The [K] of the snail tissues increased from 30 ± 10.1 mMol l^{-1} to 58 ± 11.2 mMol l^{-1} after 60 days at 85% r.h., but at 96% r.h. the increase changed from $30 \pm$ mMol l^{-1} to 42 ± 10.2 mMol l^{-1} . The oxygen consumption rate (VO_2) decreased by 66% from 60.0 ± 19.1 μl O_2 g^{-1} snail h^{-1} to 20.5 ± 16.0 μl O_2 g^{-1} snail h^{-1} after 60 days at 96% and 85% r.h. In laboratory experiments without sunlight, snails were still alive after 5 months on mud. In field experiments conducted in direct sunlight on mud, snails were dead within 24 h. At r.h. of 96%, 92%, 85%, 74%, and 57% in the laboratory, the snail survival rate was respectively 40%, 40%, 42%, 22%, and 0% after 60 days. After 60 days at 96% r.h., the snail mass loss was 36%, but only 20% at r.h. of 74%.

Key words: aestivation, oxygen consumption rate, osmotic pressure, shell permeability, tissue electrolytes.

INTRODUCTION

Survival strategies of freshwater snail (intermediate) hosts of schistosomes during periods of dry conditions were described by Cort (1919), Barlow (1933, 1935), and Precht (1939). In a review, Appleton (1978) reported that all snail intermediate hosts for bilharziasis, such as *Bulinus (Bulinus) truncatus* and *Bulinus (Physopsis) africanus*, are able to tolerate desiccation in shaded temporary water bodies that gradually dried up. This also applies to *Bulinus tropicus*, an intermediate host for paramphistomiasis, which is a trematode that produces massive and acute infections in wild grazing domestic livestock in Africa (Brown, 1994). This usually occurs at the onset of the cooler winter season in combination with a high relative humidity layer created by mud in the close vicinity of the snail. Other factors, such as temperature, exposure time, snail size, and probably shell thickness, also play a role in determining desiccation toler-

ance (Gerard, 2001). Desiccation survival of freshwater snails are of epidemiological importance, because the infection can be carried over a dry period within the desiccated snail (Hira, 1968). The Bulininae, such as *Bulinus (Bulinus) tropicus* (Krauss) and *Bulinus (Physopsis) africanus* (Krauss), are better equipped to withstand desiccation in mud for up to one year compared to the Planorbinae, such as *Biomphalaria glabrata* and *Biomphalaria pfeifferi* (Appleton, 1978; Brown, 1994; Woolhouse & Taylor, 1990). Dormant land snails may survive for six years in museum collections without food or water (Comfort, 1957; Machin, 1967). An eco-physiological study (Schmidt-Nielsen et al., 1971) on a desert pulmonate snail (*Sphincterochila boissieri*) showed that snails can survive temperatures between 50°C and 55°C with a water loss rate of 0.5 μl per day for a 4 gram snail and a mean oxygen consumption rate of 15.63 μl O_2 h^{-1} for a 4 gram snail at 35°C but only 2.62 μl O_2 h^{-1} at 15°C.

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The African bulinids have been successfully induced to aestivate in the laboratory (Annecke & Peacock, 1951; Cridland, 1967; Coles, 1969; Heeg, 1977) and in field experiments (Barlow, 1935; Coles, 1969; Shiff, 1964), but are also able to survive seasonal drying up of their natural habitats (Appleton, 1978). The field observations indicated that the snails survive better when the relative humidity is high. Field data (Shiff, 1964), however, gave no precise information as to what the tolerance limits in time were for snails exposed at precisely known different humidities. Laboratory studies by Vianey-Liaud & Lancastré (1986a, b) on adult and juvenile *Biomphalaria glabrata* showed that survival over long (16 weeks) periods occurred only when the atmosphere was saturated. Different terms have been used to describe animals exposed to dehydration conditions, which may finally result in anhydrobiosis (Stiglingh & Van Eeden, 1977), cryptobiosis, anabiosis, or anoxybiosis (Keilin, 1959; Clegg, 2001). Heeg (1977) and Brown (1994) uses the term aestivation to describe physiological quiescence, desiccation, and dormancy in freshwater snails kept out of water on a surface of damp river sand or mud.

Except for the work by Heeg (1975, 1977) on the oxygen consumption rate of *Bulinus* (*Physopsis*) *africanus* and Von Brand et al. (1957) on the physiology of *Biomphalaria glabrata* during aestivation, very little is known about the physiology and the water permeability of shells on freshwater snails during conditions of desiccation.

In this laboratory study, we hypothesized that the shell wall of *Bulinus* (*Bulinus*) *tropicus* is permeable to water, resulting in the diminution of body mass and oxygen consumption rate and thus causing an increase in haemolymph osmotic pressure and tissue sodium and potassium concentration. Radio-active labelled water ($^3\text{H}_2\text{O}$) was used to quantitatively determine shell permeability. The survival time of snails exposed at pre-determined humidities were also measured.

MATERIAL AND METHODS

Fifty full grown snails (9–10 mm) were collected at a gravel pit about 5 km from Potchefstroom adjacent to the Potchefstroom-Parys road (26°43.954'S, 27°8.109'E). This water body usually dries up at the end of the summer rain season. Another group of about

20 snails, already in a desiccated condition, was also collected. In the laboratory, the desiccated snails were placed in a breeding aquarium. After about one hour, they were actively crawling and feeding, and after five days in captivity they laid eggs. The F_1 -generations of the large snail group and the desiccated field snails were used for the experiments and cultured in a aquarium unit with 34 litre capacity (De Kock & Van Eeden, 1986). The 34 litre capacity aquarium unit, consisting of a stainless steel container suspended inside a stainless water bath, was used. Polyvinyl chloride culture baskets, 15 cm in diameter and 11 cm in height with bottoms of fine Perlon gauze with a mesh size of 45 μm , were suspended in the aquarium water kept at 26°C. Snails were kept, fed (Tetramin Conditioning Food, Tetra-Werke, Germany), and reared in these baskets. Borehole water with water hardness of 193 mg l^{-1} (as carbonate) with concentrations of Ca, Mg, and bicarbonate of 1.98, 1.97, and 2.0 mMol l^{-1} respectively at a pH of 8.2, analysed by Midvaal Water Company (Accredited Lab. No T0132), was used. The culture water from the biological filter and activated charcoal tanks were supplied by gravity feed through small bore piping to each basket from an overhead reservoir.

Before each group of 60 adult aquarium snails, earmarked for the desiccation experiments, were exposed to either 92%, 85%, 74%, or 57% relative humidity (r.h.) in air at 15°C they were kept at 96% r.h. for one week at 15°C. To do this, each snail from a group was firstly placed in a small perforated Perspex weighing vial provided with a handle. The Perspex vial, with a known mass, was then hung from a hook, cemented with epoxy resin (Araldite) onto the underside of the glass lid of an 80 ml glass weighing vial. The relative humidity of the air in each of the sealed glass weighing vials was established with saturated salts of potassium sulphate (92%), zinc sulphate (85%), sodium chloride (74%), and sodium bromide (57%) (Olivier & Barbosa, 1958; Von Brand et al., 1957). Three ml of the saturated salt solutions were placed in each glass vial. For 96% r.h., filter paper saturated with water was used. The percentage mass decrease and survival rate of each snail, exposed to the different desiccation conditions, were determined every ten days for a period of 60 days. To establish death, each snail's foot surface, under a stereo-microscope, was stimulated with a needle. If no withdrawal response

of the footpad was observed at 16 times magnification, the snail was considered dead. The remaining snails that survived the 60 day exposure periods recuperated fully when placed in aquarium water.

To determine the osmotic pressure of the haemolymph samples and the Na and K concentrations in snail tissue, samples were individually collected from snails after 15, 27, 45, and 60 days. Haemolymph was collected using the Bekius-effect (Lever & Bekius, 1965; S-Róza & Sakarov, 1989).

For the osmotic pressure and the sodium and potassium measurements, 60 snails for each measurement were respectively subjected to each of the different relative humidities. The osmotic pressure of the haemolymph in individual snails was determined with the aid of a nanoliter osmometer (Model 830, Fiske, USA). In the 12 wells of the osmometer, six wells were used for the standard (100 m Osm.kg⁻¹ water), and the other six wells used for three samples from each of two snails.

To determine the potassium and sodium concentration of the snail tissue, individual snails were placed inside a dry test tube and killed by dipping part of the test tube in boiling water for 20 seconds. The snail was taken out of the test tube, and the dead body mass pulled out of the shell over a piece of aluminium foil. The wet body mass with its adherent body fluids was dried overnight at 65°C. This method was also used to determine the dried body mass, as a percentage of the total snail mass. After determination of the dried body mass the total tissue water was calculated by subtraction and the Na and K determined with a flame photometer (EEL 227 integrating flame photometer, UK), according to Van Aardt & Coertze (1981). For the osmotic and tissue potassium/sodium measurements, at least seven snails per time interval were sacrificed for the analyses. The oxygen consumption rate (VO₂) was measured with a 14-flask Gilson-constant pressure manometric respirometer (Model GR 14, Gilson Medical Electronics, USA). The VO₂ of 20 control snails was measured in water using a Warburg-flask without a sidearm and the centre well filled with 10% KOH (Umbreit et al., 1972). The VO₂ of the experimental snails was measured at 96% and 85% r.h. in the air using 20 snails for each r.h. To do this, the bottom of the respiratory flasks were covered with 5 ml water (96% r.h.) or a saturated 5 ml solution of ZnSO₄ (85% r.h.) (Von Brand et al. 1957). On top of the empty centre well

of the respiratory flask, each desiccated snail was kept in a conically shaped polyethylene mesh. The side arm of the flask contained a strip of filter paper dipped into 10% KOH. All measurements were made at 15°C and the VO₂ expressed in µl oxygen per gram live snail mass (with shell) per h. After the VO₂ measurements, the snails were transferred back to the glass vial set up for the different relative humidities. Sodium and potassium in the tissues as well as the osmotic pressure of the haemolymph and the VO₂ were measured for snails exposed in air only at 96% and 85% r.h.

To determine the permeability of the shell for tritiated water (³H₂O), 20 adult snails were killed in boiled water. The freshly cleaned shells were dried for two h at 65°C. Under a stereo microscope, the mouth opening of each shell was heat sealed with a 1.5–2.0 mm thick layer of a aquarium sealant wax (Hykro Leak Stopper, Hykro, Denmark) using a hot spatula. Tritiated water (30 µl) with an activity of 3.7 x 10⁴ Bq per 50 µl was injected with a Hamilton syringe (Hamilton Co, USA) through the wax layer into the shell and the needle opening hot sealed. The fixed Hamilton needle was provided with a side opening near the needle tip to be able to prevent needle blockage during the injection. Each sealed shell was then placed into the Perspex vial, and hung in the 80 ml capacity sealed glass vial at a r.h of 96% in air for 24 h as described above. After the 24 h exposure period, a 2 ml sample of the water at the bottom of the 80 ml capacity glass vial was collected and counted for radioactivity (Van Aardt & Coertze, 1981). The amount of water that permeated through the shell wall after 24 h was determined by a radioisotope dilution method (Morris & Van Aardt, 1998). To detect possible leakage of the wax sealed shell mouth and to determine the permeability of the shell to high molecular substances, 30 µl radioactive inulin (Phelps, 1965), labelled with ¹⁴C (4.1 x 10⁴ Bq per 50 µl) was injected into another 20 sealed shells. The radioactive inulin solution sampled from the water at the bottom of the vial was counted in the same manner as described for tritiated water. As controls (in place of *B. tropicus* shells), 20 mini glass vials with 500 µl capacity was used. A 2 mm thick seal of the aquarium sealant wax was melted over the mini vial mouth as described for empty snail shells. A solution of 30 µl radioactive inulin was then injected and the vial mouth sealed and treated in the same manner described for the sealed empty shells.

Ten shells from the field population at Potchefstroom and the F_1 -generation in the laboratory were cleaned and the thickness of the shell wall measured at the mouth opening by a digital calliper (Mitutoyo, Model CD-8; 0.01 mm). Ten shells from a soft water area (Rietfontein Dam, Pretoria) were also measured for shell thickness. For each population the carbonate hardness of the water was analysed by a Merck Aquamerck Analysis Kit 8048. For the laboratory experiments, all the measurements on snails were done without exposure to sunlight. To find out if non-sunlight conditions could influence the desiccation physiology of laboratory snails two field experiments, with and without sunlight was undertaken. Twenty specimens of *B. tropicus* were, for each experiment placed in a glass container (7 cm wide and 5 cm high) filled with 3 cm of soft mud (obtained from the gravel pit) covered by one cm of water to simulate field conditions (Cridland, 1967). The glass vials were placed into moist soil of 5 cm depth. Air temperature, in sunlight, was 25–27°C. Without sunlight, snails were kept in the same glass containers, with mud and water at 15–20°C.

RESULTS

For the experiments we used F_1 -generation snails bred in the laboratory and not snails collected in the field. It was shown (Heeg, 1975) that field snails kept in aquarium water are subjected to physiological shock resulting in high mortalities before starting the desiccation experiments. In the first field experiment, with direct sunlight exposure, all the water evaporated within 5 to 7 days, and the snails were dead within 24 h. No attempt was made by the snails to dig or crawl into the mud. In the second field experiment without sunlight, snails were still alive after 5 months. The snails crawled into the mud with only a small part of the shell exposed with hardened mud sealing off the shell opening. Specimens of *B. tropicus* kept in air at all the five different relative humidities in the sealed glass containers were deeply contracted into the shell with the foot surface covered with what appears to be a thin layer of dried mucus.

The water carbonate hardness for the Potchefstroom habitat was 193 mg l⁻¹ and for the Rietvlei Dam 103 mg l⁻¹. The mean shell

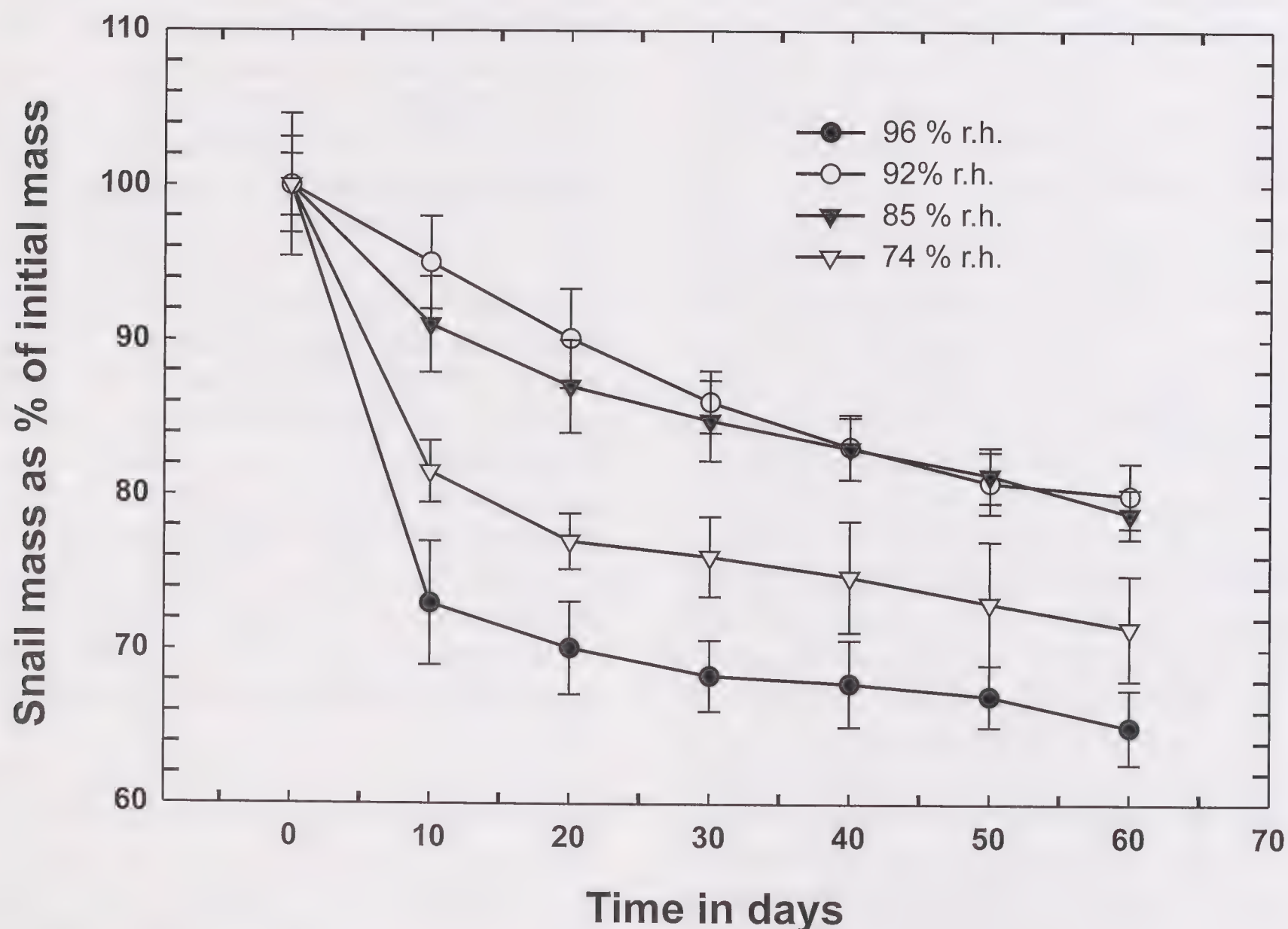


FIG. 1. Percentage decrease in snail mass during the 60 days exposure to different relative humidities (r.h.). Sixty snails were used for each r.h. Error bars denote the standard deviation from the mean.

thickness of the snails at the Potchefstroom population living at the gravel pit in hard water together with snails reared in the laboratory was $95.0 \pm 2.0 \mu\text{m}$ and the shells from the softwater habitat at Rietvlei Dam was $27 \pm 1.0 \mu\text{m}$.

In general, the largest decrease of nearly 30% in mass, for the five different r.h. investigated, occurred within the first 15 days for the 96% and 74% r.h. exposed snails. After this period, the snail mass decreased, on average less than 10% for the next 45 days when exposed to the different r.h. (Fig. 1).

After 60 days at 96%, 92%, 85%, 74%, and 57% r.h. exposure, the percentage survival was 40%, 40%, 42%, 22%, and 0% snails, respectively. Between 37% and 42% of the snails exposed to 96%, 92%, and 85% r.h. were still alive after 60 days, but at 74% r.h., 78% were dead, and at 57% r.h., all were dead 16 days after the onset of the experiment.

Sodium concentration in the snail tissue increased 79% to 68 mMol l^{-1} for snails exposed at 85% r.h., and 37.5% to 55 mMol l^{-1} at a r.h. of 96% for 60 days. For tissue potassium, the increase was 33% from 30 to 42 mMol l^{-1} at 96% r.h., and 83% from 30 to 58 mMol l^{-1} at 85% r.h.% (Fig. 2).

A threefold increase in the haemolymph osmotic pressure (Fig. 3) from 100 to 300 mOsm l^{-1} water was found for snails at 85% r.h. exposed for 60 days. For 96% r.h., exposed snails the increase was nearly two and a half times above the normal osmotic pressure value of 100 mOsm l^{-1} water.

From day 1 to day 60, the VO_2 decreased by 66% from 60 microlitres per gram snail (including the shell) per h to 20.5 microlitres per gram snail per h (Fig. 4). If the VO_2 is expressed per mg dried body mass (without the shell) (Van Aardt et al., 2003), it decreases from $0.627 \mu\text{l O}_2 \text{ mg}^{-1} \text{ dried body mass hr}^{-1}$ to $0.209 \mu\text{l O}_2 \text{ mg}^{-1} \text{ dried body mass hr}^{-1}$. The VO_2 expressed in these units allows physiologists to compare oxygen consumption rates between pulmonates with and without shells. The decrease in the amount of oxygen consumed measured for 96% and 85% r.h. exposed snails after 60 days are the same (Fig. 4). Large variations in VO_2 , however, were encountered between individual snails.

The tritiated water, as a percentage of the amount injected ($30 \mu\text{l}$) escaping through the shell wall, was 5.79% (Table 1), or $1.74 \mu\text{l}$ in 24 h. It was assumed that a snail with a snail

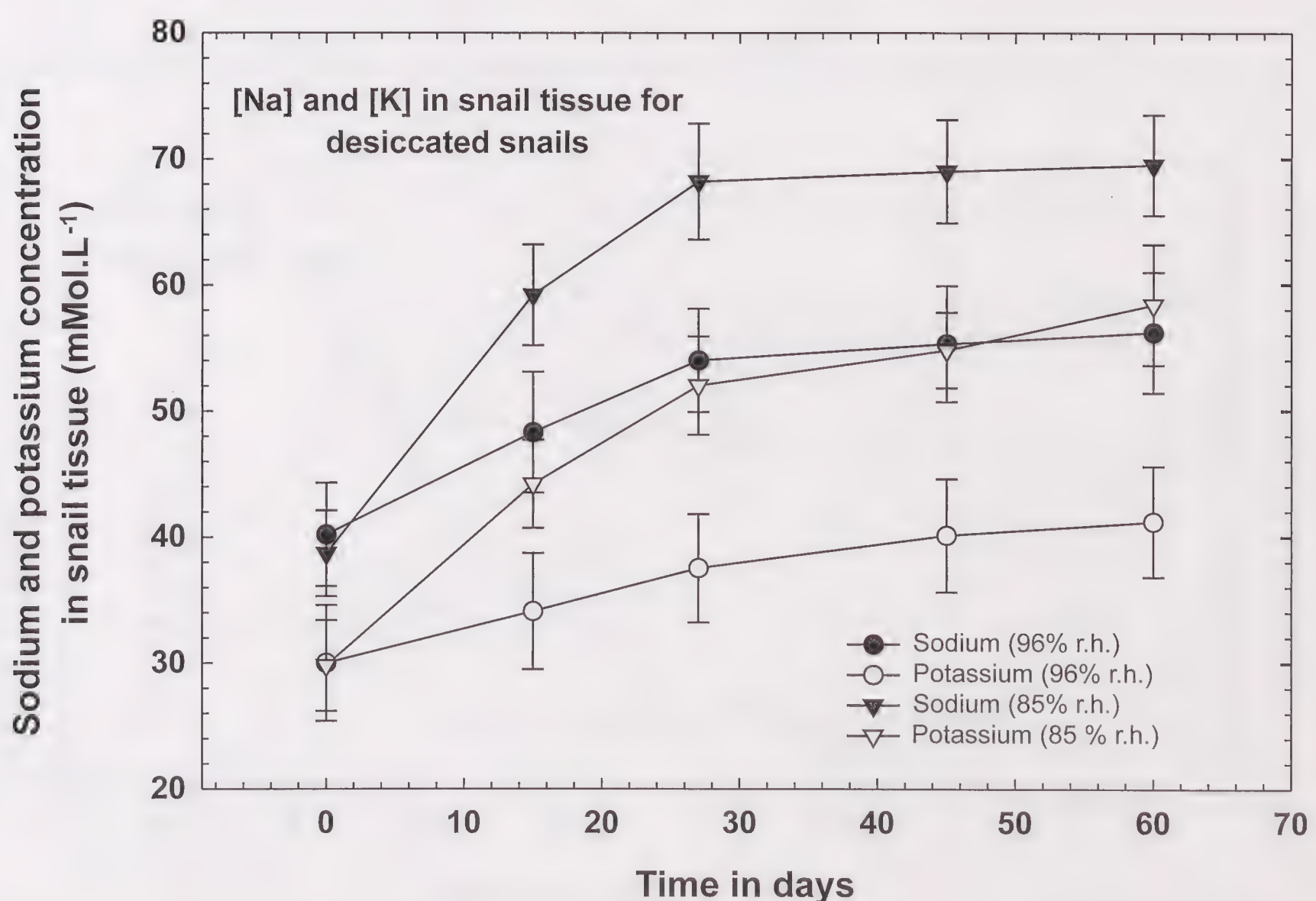


FIG. 2. The sodium and potassium concentrations in the tissues of snails exposed for 60 days at different relative humidities (r.h.). The number of snails alive after the exposure was 7, 7, 4, and 4 specimens for each r.h. Sixty snails were used for each r.h. Error bars denote the standard deviation from the mean.

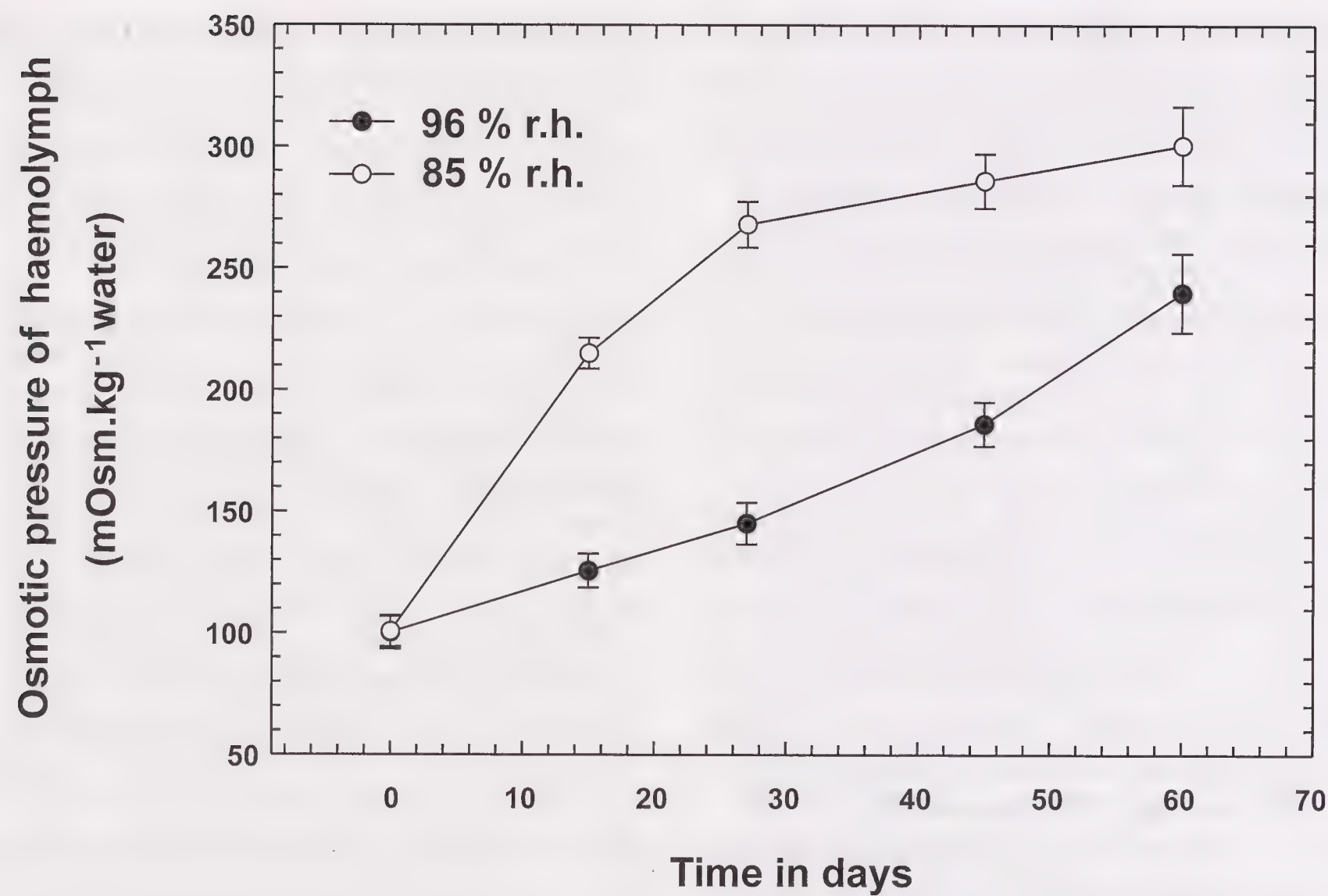


FIG. 3. The osmotic pressure of the haemolymph for each group of 40 snails exposed to 96% or 85% relative humidity. At least seven snails were used for each determination. Error bars denote the standard deviation from the mean. The number of snails alive after the exposure period was 5 specimens for each r.h.

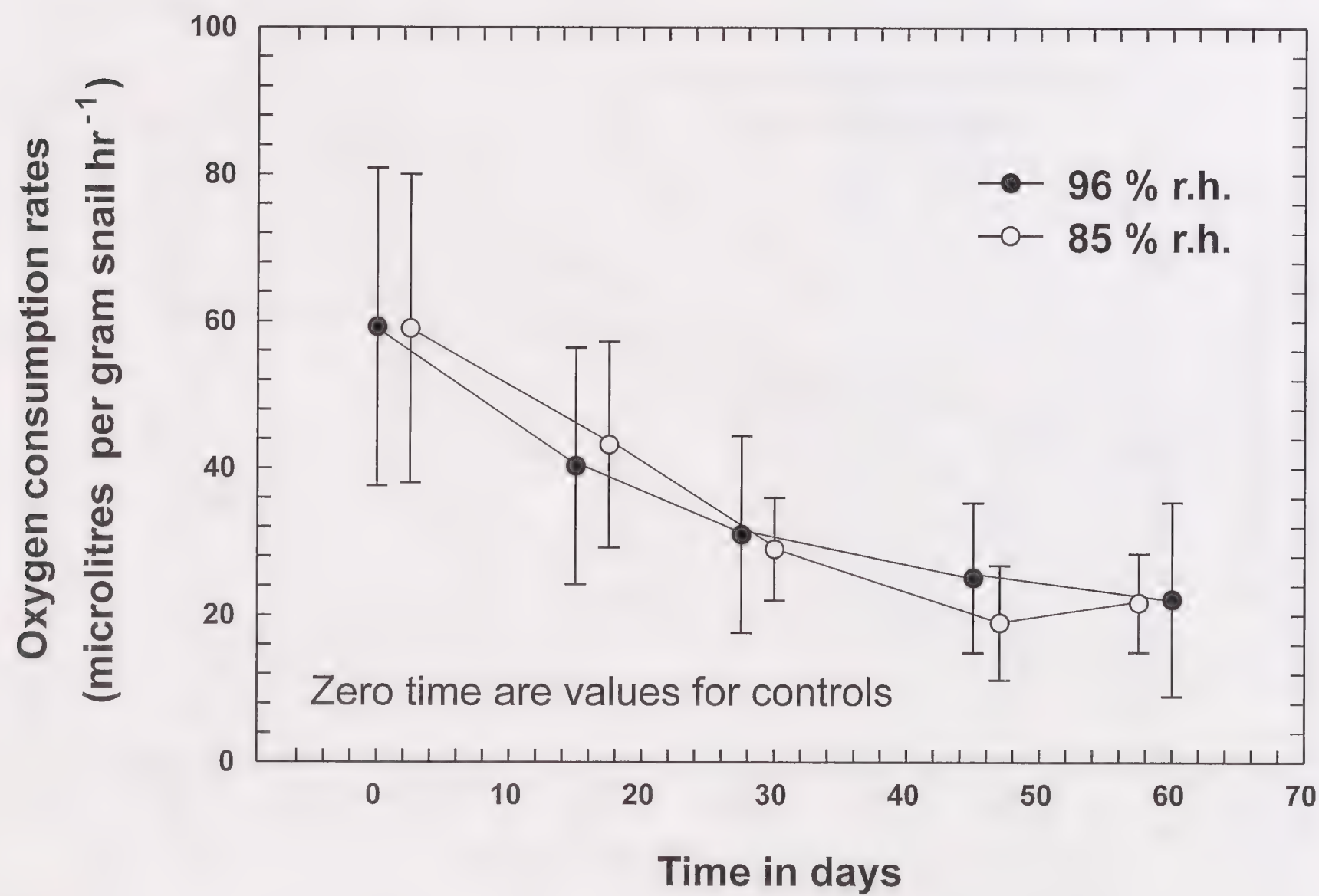


FIG. 4. Oxygen consumption rates at 15°C for aestivated snails at 96% and 85% r.h. over a period of 60 days.

TABLE 1. Tritiated water and Inulin-¹⁴C found in the water of the 80 ml sealed glass vials 24 h after injection into the shells of *Bulinus tropicus* and the mini glass vials. Dpm: disintegrations per minute; ±: Standard deviation from the mean; *Data not used in the calculations (see text).

Shell no	Dpm of ³ H ₂ O found in the water	% of ³ H ₂ O found in the water	Shell no	Dpm of inulin found in the water	% of Inulin found in the water	Mini vial no	Dpm of inulin found in the water	% of inulin found in the water
1	2273	0.90	1	73	0.13	1	0	0
2	9783	3.86	2	233	0.43	2	0	0
3	12263	4.84	3	8603*	15.70*	3	0	0
4	78460	30.94*	4	463	0.44	4	0	0
5	86573	34.13*	5	133	0.24	5	4700*	8.58*
6	7398	2.92	6	16460*	30.03*	6	328	0.60
7	25803	10.17	7	0	0	7	153	0.28
8	7548	2.98	8	135	0.25	8	0	0
9	16936	6.69	9	5	0.01	9	0	0
10	28218	11.13	10	0	0	10	0	0
11	21033	8.29	11	0	0	11	270	0.49
12	19238	7.59	12	108	0.2	12	5	0.01
13	2640	1.04	13	325	0.59	13	5	0.01
14	2433	1.35	14	575	1.05	14	3	0.01
15	14675	5.79	15	183	0.33	15	10	0.01
16	16130	6.36	16	0	0	16	35	0.02
17	15888	6.26	17	123	0.22	17	15	0.01
18	25760	8.16	-	-	-	18	43	0.02
19	24850	9.10	19	11	0.02	19	5	0.01
						20	33	0.02
		5.79 ± 3.20			0.37 ± 0.29			0.12 ± 0.20

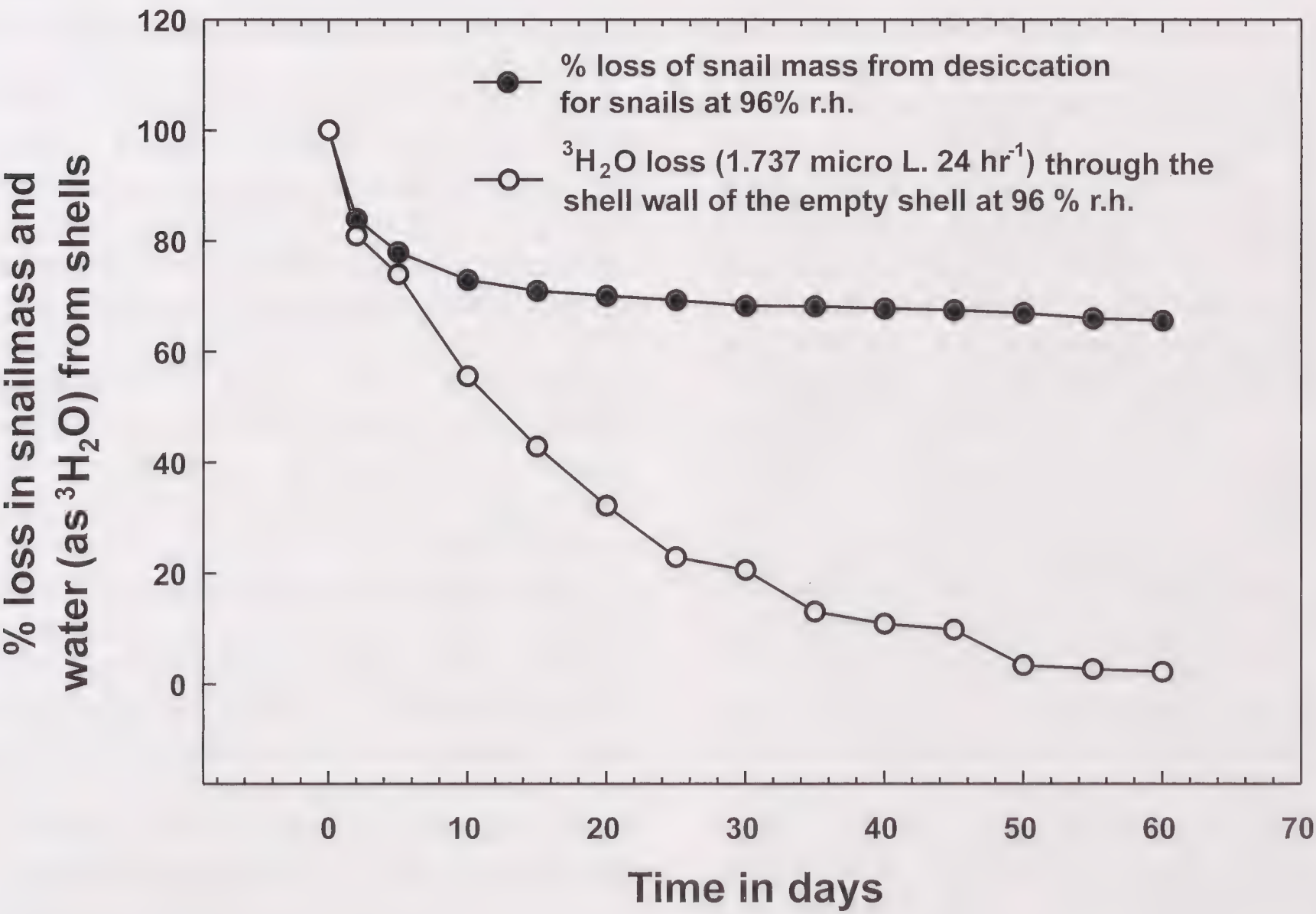


FIG. 5. Measured loss of snail mass during desiccation and calculated loss of tritiated water through the wall of empty sealed shells after 60 days.

body mass of 0.0917 gram would have a shell volume of 91.7 μl . With a daily loss of 1.74 μl water, the shell would be empty of water after about 60 days (Fig. 5). When tritiated water values are compared with the high molecular mass compound, inulin, only 0.37% of inulin was found in the water of the sealed glass vial and only 0.12% of inulin found in the water of the sealed 500 μl mini glass vial. The very high inulin radioactive values found in the water of the inulin injected shells (Table 1, shown with an asterisk) are due to leakages through the sealant, because similar leakages were found with the sealed mini glass vials when injected with inulin. Therefore, data annotated with asteriks were not used to evaluate the results. In Figure 5, the percentage loss in snail mass is compared with the calculated cumulative tritiated water loss through the shell wall kept at 96% r.h. The results indicate that the tritiated water injected into the empty shell through the sealed off shell mouth was all lost after 60 days. In comparison, the water component in the desiccated snail, withdrawn inside the shell, decreased by only about 40% during the 60 days exposure to 96% r.h.

DISCUSSION

Our measurements with tritiated water clearly show that the shell wall of *Bulinus* (*Bulinus*) *tropicus* is permeable to water. This cannot be ascribed to leakages of $^3\text{H}_2\text{O}$ through the sealant that covers the shell mouth or by other phenomena. So far as we know, this is the first attempt to measure the permeability of the shell wall for water of a freshwater snail. Compared with molluscs with an outer layer of periostracum, middle prismatic layer and a thick inner nacreous layer of more than 1 mm in thickness (Nakahara & Bevelander, 1971), the shell walls of the African basommatophorans are relatively thin. The ultrastructure and cytochemistry of the shell wall in a related basommatophoran, *Biomphalaria glabrata* (Peters, 1972; Bielefeld et al., 1993a) consists of an 1.6 μm thick outer layer or periostracum composed almost entirely of scleroprotein together with some bound carbohydrates and also chitin (Hunt & Oates, 1978). The inorganic part of the shell consists mainly of both organic matrix materials and CaCO_3 as the inorganic crystalline substance (Bielefeld et al., 1993b). Nothing is known about the water permeability ability of

these two layers. In *B. glabrata* reared in the laboratory, the total shell wall is 140 ± 0.02 μm thick (Van Aardt, personal observation) compared to 95 μm for *B. tropicus*. The shell walls of *B. tropicus* grown in the Rietvlei Dam with its soft water are about three times thinner compared with snails grown in the two hard water habitats at Potchefstroom. From the permeability data, it can be deduced that the thicker shell walls of the Potchefstroom populations, growing in hard water, should be less permeable to water and thus more resistant to conditions of desiccation. It is interesting to note (Fig. 5) that less water is lost in the living snail during aestivation compared with the empty shell, partly filled with radioactive water. A possible explanation is that the peripheral snail tissues, especially the mantle and muscular foot, are effective barriers that prevent water evaporation during the tight contraction into the shell of the soft body parts.

The relative large loss in water for the 96% r.h. exposed snails compared to the 85% and 92% r.h. exposed snails (Fig. 1) could be explained by the activity of the snails during the exposure period. The soft body parts of these snails are observed to periodically extend out from their shells and in the process expose the wet body surface to evaporative water loss into the aerial space of the sealed container.

The oxygen uptake rates for *B. tropicus* are in good agreement with those reported for other basommatophorans (Von Brand et al., 1957; Van Aardt et al., 2003). After 60 days of desiccation at 15°C, the VO_2 was 0.209 ± 0.07 $\mu\text{l O}_2 \text{ mg}^{-1}$ dried snail hr^{-1} , which is nearly the same as the 0.33 $\mu\text{l O}_2$ per mg dried snail tissue per h found for *Bulinus* (*Physopsis*) *africanus* after 21 days in aestivation at 15°C (Heeg, 1977). Evidence from the literature on VO_2 data and metabolite levels (Schmidt-Nielsen et al., 1971; Heeg, 1977) suggests that during desiccation snails use stored reserves sparingly even at high temperatures, with lipids, carbohydrates, and proteins metabolised in equal amounts.

The decrease in respiration rate and body mass, with a concomitant increase of the haemolymph [Na], [K] and osmotic pressure, during the 60 days exposure of *B. tropicus* in air is indicative of the ability of *B. (B.) tropicus* to survive conditions of desiccation. Most species of *Bulinus* living in African water bodies have the ability to aestivate, which may have contributed to their success as carriers of schistosomes and other waterborne parasites

(Brown, 1994). Our oxygen consumption rate measurements on *B. tropicus* are in good agreement with those of Coles (1969) on *Bulinus nasutus*, and our snail mass decrease determinations agree with those of Oliver & Barbosa (1958) on *B. glabrata* using field collected snails. However, the physiology of *B. (B.) tropicus* during desiccation indicates that the adaptations these snails made cannot be compared to true cryptobiotic or anhydrobiotic animals, such as nematodes, copepods, tardigrades, and brine shrimps, for which oxygen uptake rates are not measurable and body water is almost totally lost (Clegg, 2001).

During the seven days exposure to 96% r.h. in air, all the snail groups were active and showed crawling movements of the exposed footpad. After this period, movements were only observed for snails kept at the 92% r.h. for day 8 to 9, after which deep withdrawal into the shells occurred. This behaviour was also found for *Pila virens* (Meenakshi, 1956), *Pomacea lineata*, *P. depressa* (Little, 1967), and for *Bulinus (Physopsis) africanus* (Heeg, 1977). Brown (1994) classified *Bulinus tropicus* as a good aestivator, together with five other bulinids living in ephemeral waters that may not be defined as aquatic habitats during the dry season. At 12–14% relative humidity exposure of *Heliosoma trivolvis*, Gallo et al. (1984) found a LD₅₀ at 20–22°C after 45 h exposure, whilst the haemolymph osmolality increased from 99 mOsm.kg⁻¹ H₂O to 173 mOsm.kg⁻¹ H₂O.

The high rate of water loss for the five different r.h. groups, kept at 96% r.h. during the first 10 days, could be explained by the relatively high activity of the snails resulting in the loss of water between the shell and snail body. This was followed by a much slower rate of water loss for snails kept from day 10 at 92%, 85%, 74%, and 57% r.h. (Fig. 1). The water loss originates mainly from body water inside the snail (Komiya & Hasimoto, 1958).

The three-fold increase of the of the haemolymph osmotic pressure and the concomitant increase of the tissue [Na] and [K], together with a loss in body mass, indicates water loss as the major factor in the desiccation condition of the snail. However, little is known how extensively freshwater snails can tolerate increases of electrolytes in the tissues or in the haemolymph. Water loss at 12–14% r.h results in an increase of the haemolymph osmotic pressure in *Heliosoma trivolvis* from 100 mOsm l⁻¹ to 173 ± 10 mOsm l⁻¹ within 18 h (Gallo et al., 1984).

Our results thus show that *B. tropicus* can adapt physiologically to desiccation in the laboratory and most probably in the field, but it is still uncertain whether biochemical and biophysical mechanisms are involved in freshwater snails in which polyhydroxy compounds such as trehalose play a major part in well-adapted anhydrobiotic organisms (Clegg, 2001). For freshwater snails, induction to the desiccation process, is subject to low temperatures, initial high r.h. with no surrounding water and applicable to full grown snails. *Bulinus (B.) tropicus* and *B. truncatus* are more resistant to desiccation than *Biomphalaria* species because of their mud burrowing ability (Ghandour, 1987). The water permeability of the shell wall in freshwater snails should be systematically investigated to establish the magnitude of this permeability. It is known that both genetic and mechanical factors are implicated in the ability of freshwater snails to survive out of water (Brown, 1994).

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